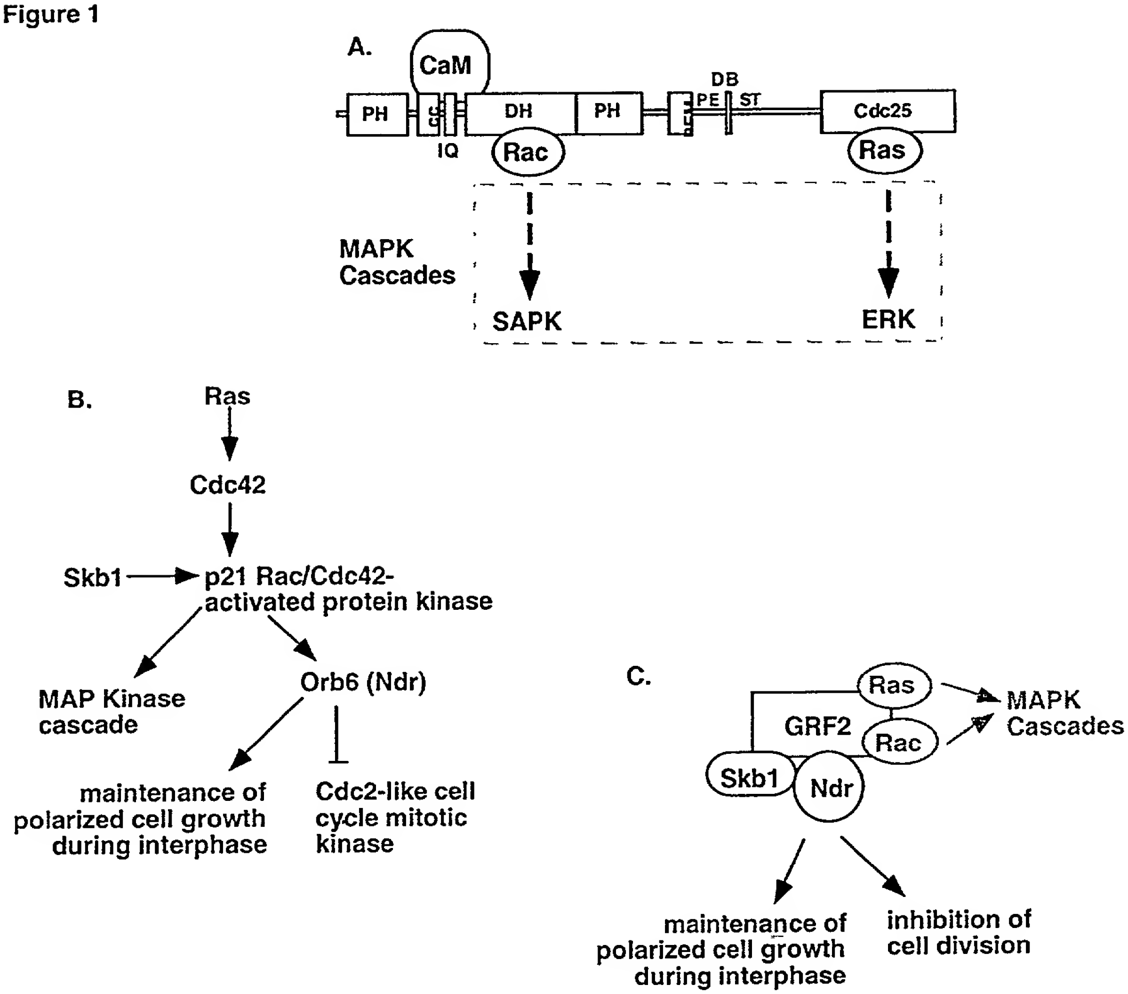
Figure 1



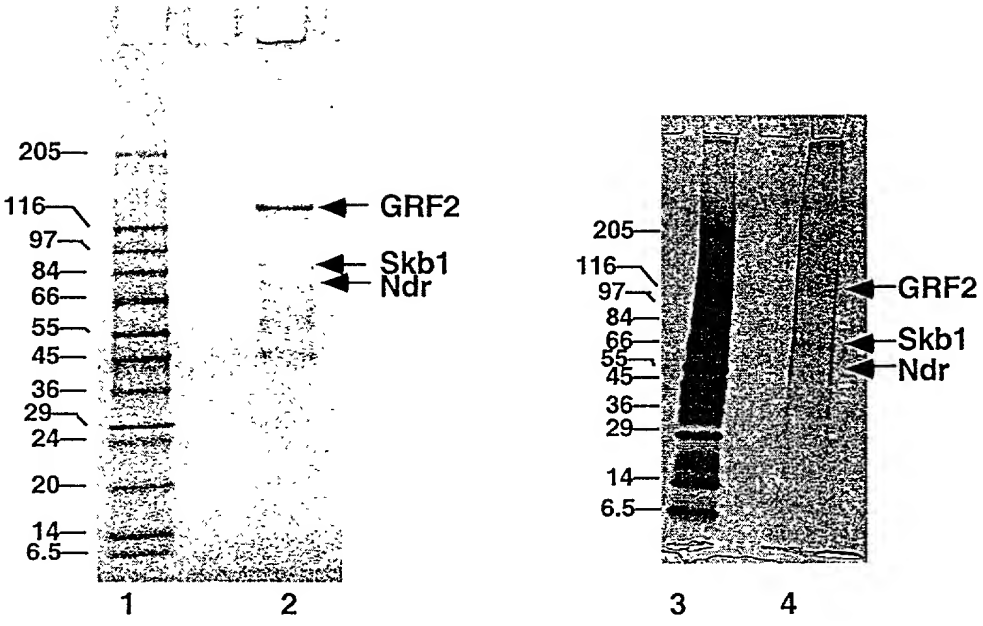
<u>Legend to Fig. 1</u>

A. Ras-GRF2: schematic diagram showing domain structure in amino-to-carboxy orientation. GRF1 is similar, but lacks an obvious DB sequence. Domain definitions: PH, pleckstrin homology; cc, coiled coil; IQ, ilimaquinone domain; DH, Dbl homology; REM, Ras exchanger motif; PEST, rich in proline/glutamate/serine/threonine residues; DB, destruction box; Cdc25, Cdc25p homology. The DB motif is located within the PEST region. Proteins known to interact with GRF2 are indicated: Ras, Rac and calmodulin (CaM). Ref. Fam et al. 1997.

B. A genetic pathway in fission yeast (after Verdi et al. 1998) containing gene products corresponding to human proteins described in this invention as forming part of a complex of interacting proteins.

C. A protein complex isolated from human 293 epithelial cells that contains GRF2, Skb1 and Ndr—homologs of yeast proteins known to inhibit mitosis and maintain polarized cell growth. Shown in grey are known GRF2 interactions with proteins Ras and Rac and the coupling of these interactions to MAPK pathways.

Figure 2



Legend to Fig. 2

Colloidal Coomassie Blue (on the left) and silver (on the right) stained SDS-polyacrylamide gels containing the GRF2 protein complex (lanes 2 and 4). Lanes 1 and 3 contain protein standards of the indicated molecular masses (in kilodaltons). Arrows indicate the positions of the indicated proteins. Their unambiguous identities were assigned following excision of these stained bands, digestion with trypsin, analysis by mass spectrometry, and protein database searching.

Figure 3A.

Peptide 40 (num. hits = 92)

(1.0) GTVLDQVPVNPSLYLIK (17.0)

Symbol	Mass	a	a - 17	b	b - 17	y	y - 17
G, Gly	57.021	30.034	13.032	58.029	41.027	1856.053	1839.051
T, Thr	101.048	131.082	114.079	159.077	142.074	1799.032	1782.029
V, Val	99.068	230.150	213.148	258.145	241.143	1697.984	1680.981
L, Leu	113.084	343.235	326.232	371.229	354.227	1598.916	1581.913
D, Asp	115.027	458.261	441.259	486.256	469.254	1485.832	1468.820
Q, Gln	128.059	586.320	569.317	614.315	597.312	1370.805	1353.802
V, Val	99.068	685.388	668.386	713.383	696.381	1242.746	1225.743
P, Pro	97.053	782.441	765.439	810.436	793.433	1143.678	1126.675
V, Val	99.068	881.510	864.507	909.505	892.502	1046.625	1029.622
N, Asn	114.043	995.553	978.550	1023.547	1006.545	947.557	930.554
P, Pro	97.053	1092.605	1075.603	1120.600	1103.598	833.514	816.511
S, Ser	87.032	1179.637	1162.635	1207.632	1190.630	736.461	719.458
L, Leu	113.084	1292.721	1275.719	1320.716	1303.714	649.429	632.426
Y, Tyr	163.063	1455.785	1438.782	1483.780	1466.777	536.345	519.342
L, Leu	113.084	1568.869	1551.866	1596.864	1579.861	373.282	356.279
I, Ile	113.084	1681.953	1664.950	1709.948	1692.945	260.197	243.195
K, Lys	128.095	1810.048	1793.045	1838.043	1821.040	147.113	130.111

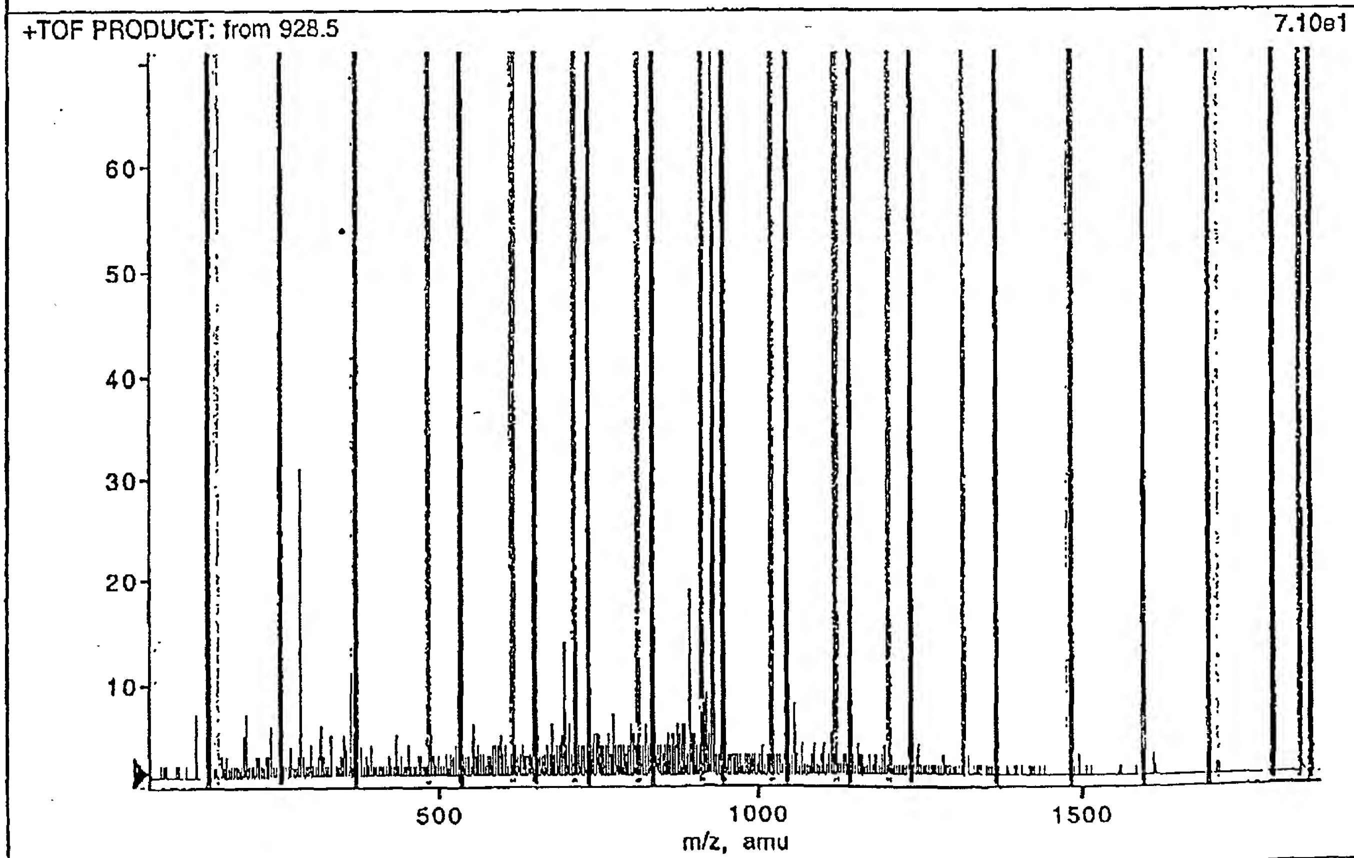


Figure 3B.

Peptide 39 (num. hits = 57)

(1.0) VSALEVLPDR (17.0)

Symbol	Mass	a	a - 17	b	b - 17	y	y - 17
V, Val	99.068	72.081	55.079	100.076	83.074	1098.616	1081.613
S, Ser	87.032	159.113	142.111	187.108	170.106	999.547	982.545
A, Ala	71.037	230.150	213.148	258.145	241.143	912.515	895.513
L, Leu	113.084	343.235	326.232	371.229	354.227	841.478	824.476
E, Glu	129.043	472.277	455.274	500.272	483.269	726.394	711.392
V, Val	99.068	571.346	554.343	599.340	582.338	599.352	582.349
L, Leu	113.084	684.430	667.427	712.424	695.422	500.283	483.281
P, Pro	97.053	781.482	764.480	809.477	792.475	387.199	370.196
D, Asp	115.027	896.509	879.507	924.504	907.502	290.146	273.144
R, Arg	156.101	1052.610	1035.608	1080.605	1063.603	175.119	158.117

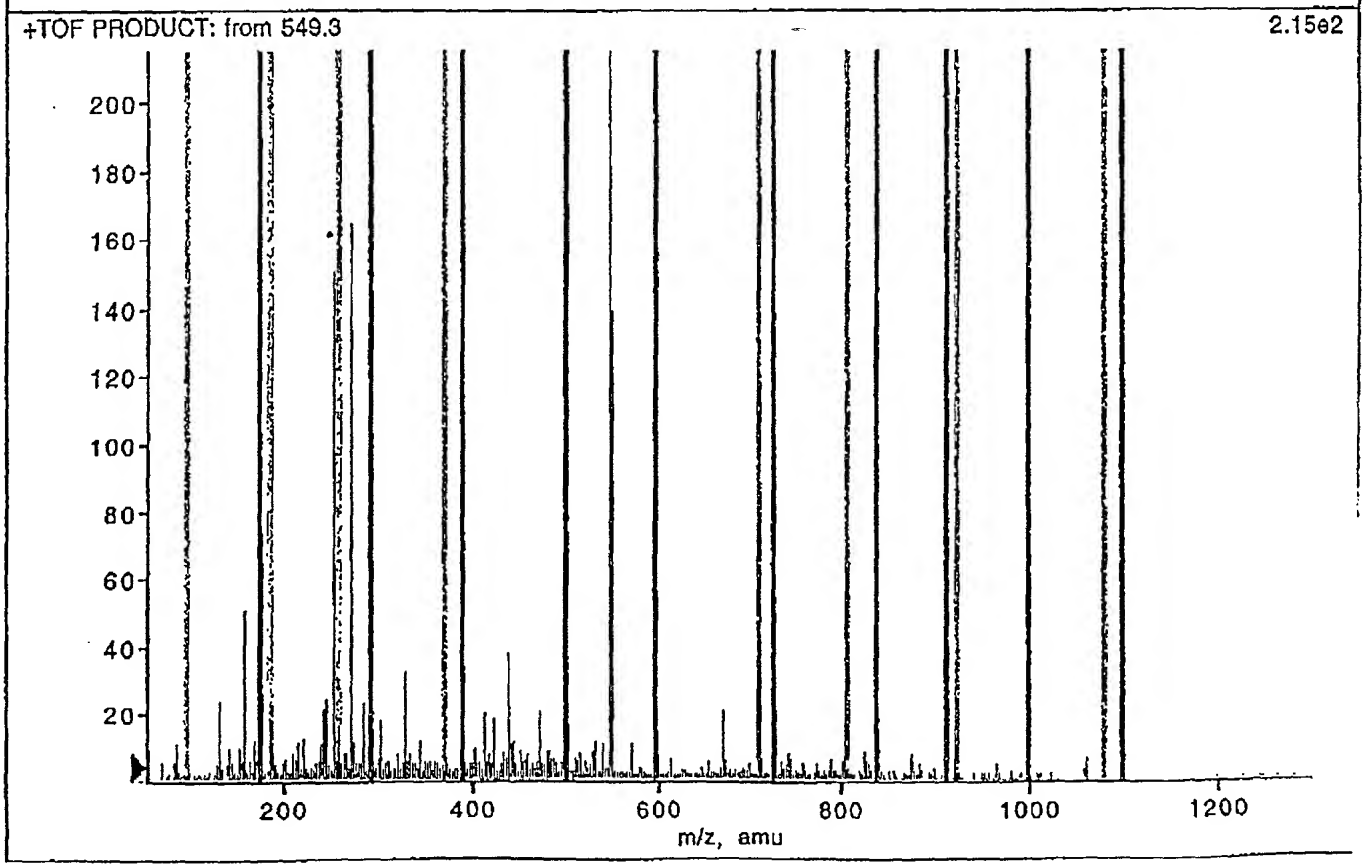


Figure 4A

Peptide 69 (num. hits = 59)

(1.0) LVTDEDVFPTK (17.0)

Symbol	Mass	a	a - 17	b	b - 17	y	y - 17
L, Leu	113.084	86.097	69.094	114.092	97.089	1263.647	1246.645
V, Val	99.068	185.165	168.163	213.160	196.158	1150.563	1133.560
T, Thr	101.048	286.213	269.210	314.208	297.205	1051.495	1034.492
D, Asp	115.027	401.240	384.237	429.235	412.232	950.447	933.444
E, Glu	129.043	530.283	513.280	558.277	541.275	835.420	818.418
D, Asp	115.027	645.310	628.307	673.304	656.302	706.378	689.375
V, Val	99.068	744.378	727.375	772.373	755.370	591.351	574.348
F, Phe	147.068	891.446	874.444	919.441	902.439	492.282	475.279
P, Pro	97.053	988.499	971.496	1016.494	999.491	345.214	328.211
T, Thr	101.048	1089.547	1072.544	1117.542	1100.539	248.161	231.158
K, Lys	128.095	1217.642	1200.639	1245.637	1228.634	147.113	130.111

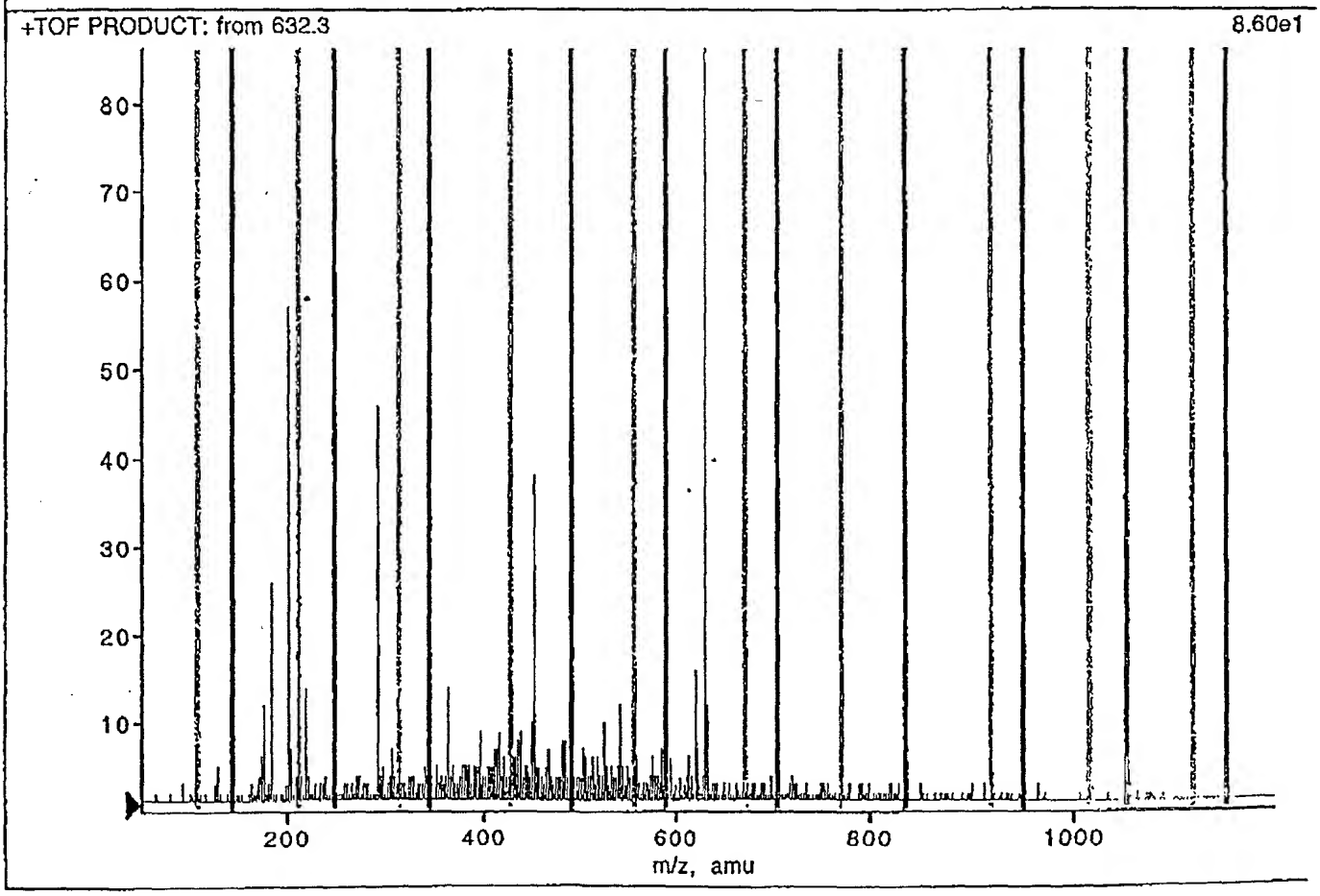
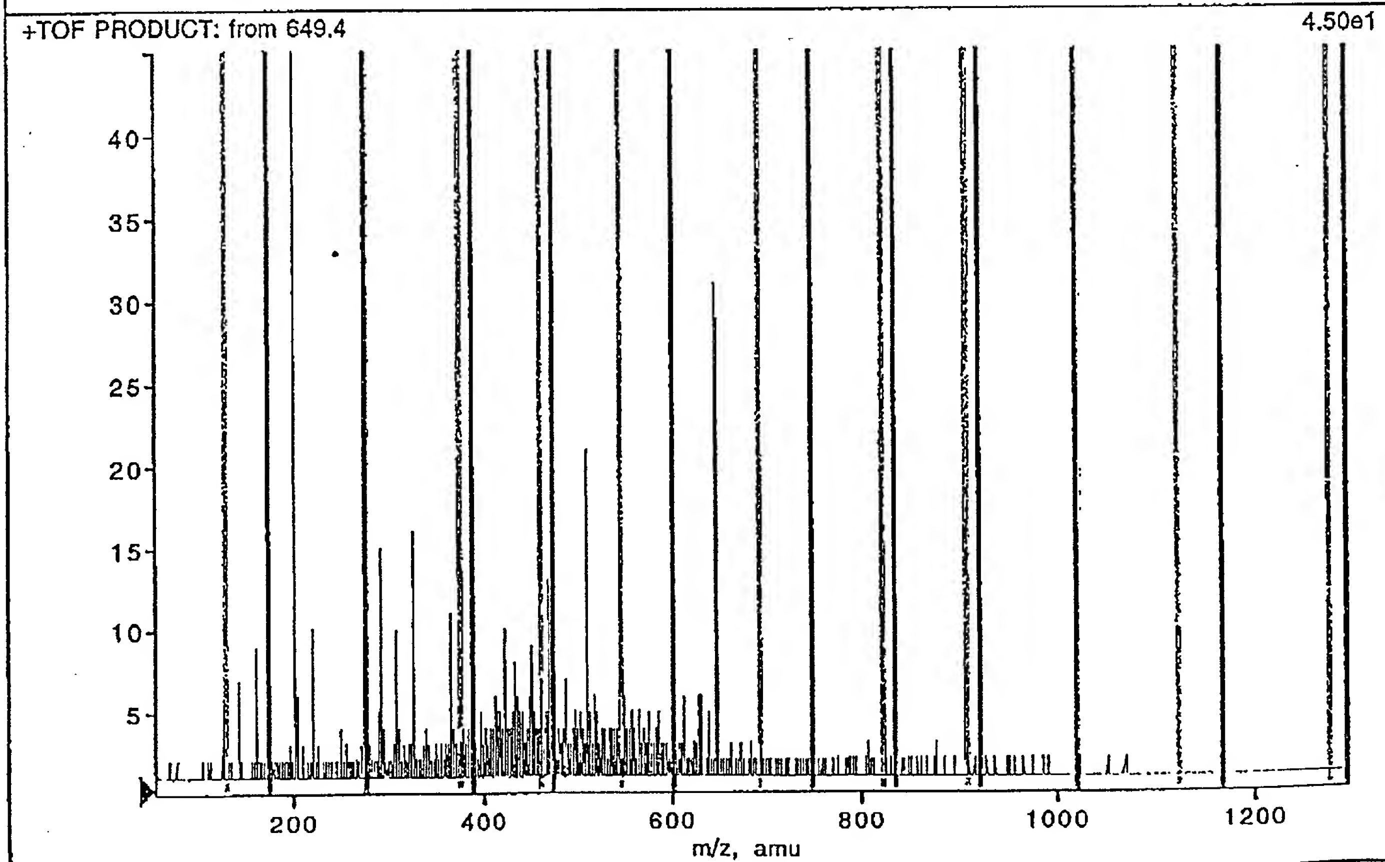


Figure 4B

Peptide 71 (num. hits = 59)

(1.0) EFPSSFESLVR (17.0)

Symbol	Mass	a	a - 17	b	b - 17	y	y - 17
E, Glu	129.043	102.056	85.053	130.050	113.048	1297.643	1280.640
F, Phe	147.068	249.124	232.121	277.119	260.116	1168.600	1151.597
P, Pro	97.053	346.177	329.174	374.172	357.169	1021.532	1004.529
S, Ser	87.032	433.209	416.206	461.204	444.201	924.479	907.476
S, Ser	87.032	520.241	503.238	548.236	531.233	837.447	820.444
F, Phe	147.068	667.309	650.307	695.304	678.301	750.415	733.412
E, Glu	129.043	796.352	779.349	824.347	807.344	603.346	586.344
S, Ser	87.032	883.384	866.381	911.379	894.376	474.304	457.301
L, Leu	113.084	996.468	979.465	1024.463	1007.460	387.272	370.269
V, Val	99.068	1095.536	1078.534	1123.531	1106.528	274.188	257.185
R, Arg	156.101	1251.637	1234.635	1279.632	1262.630	175.119	158.117



1 MRKETPPPLV PPAAREWNLP PNAPACMERQ LEAARYRSDG ALLLGASSLS
51 GRCWAGSLWL FKDPCAAPNE GFCSAGVQTE AGVADLTWVG ERGILVASDS
101 GAVELWELDE NETLIVSKFC KYEHDDIVST VSVLSSGTQA VSGSKDICIK
151 VWDLAQQVVL SSYRAHAAQV TCVAASPHKD SVFLSCSEDN RILLWDTRCP
201 KPASQIGCSA PGYLPTSLAW HPQQSEVFVF GDENGTVSLV DTKSTSCVLS
251 SAVHSQCVTG LVFSPHSVPF LASLSEDCSL AVLDSSLSEL FRSQAHRDFV
301 RDATWSPLNH SLLTTVGWDH QVVHHVVPTE PLPAPGPASV TE

Fig 5. Full-length protein sequence for EST6593318/EST5339315 protein. Peptides used to identify EST6593318/EST5339315 protein are underlined, or double underlined for adjacent peptides. The full-length cDNA was cloned by PCR amplification with a specific primer for the 5' end of EST6593318 and an oligo dT primer. The predicted protein contains 6 WD40 repeats in the centre of the molecule and unique N- and C-terminal sequences.

Protein Translation of MOB-related protein EST GI 705582

1 HHLGVLHRRD VSDDGRVHNK YYWYDERGKK VKCTAPQYVD FVMSSVQKLV TDEDVFPTKY

61 GREFPSSFES LVRKICRHLF HVLAH

MOB-related Hypotetical Protein GI 8922671

1 MSFLFSSRSS KTFKPKKNIP EGSHQYELLK HAEATLGSGN LRQAVMLPEG EDLNEWIAVN

61 TVDFFNQINM LYGTITEFCT EASCPVMSAG PRYEYHWADG TNIKKPIKCS APKYIDYLMT

121 WVQDQLDDET LFPSKIGVPF PKNFMSVAKT ILKRLFRVYA HIYHQHFDSV MQLQEGAHLN

181 TSFKHFIFFV QEFNLIDRRE LAPLQELIEK LGSKDR

Spindlin GI 5730065

1 MQAMLEVSAN MMKKRTSHKK HRSSVGPSKP VSQPRRNIVG CRIQHGWKEG NGPVTQWKGT

61 VLDQVPVNPS LYLIKYDGFD CVYGLELNKD ERVSALEVLP DRVATSRISD AHLADTMIGK

121 AVEHMFETED GSKDEWRGMV LARAPVMNTW FYITYEKDPV LYMYQLLDDY KEGDLRIMPD

181 SNDSPPAERE PGEVVDSLVG KQVEYAKEDG SKRTGMVIHQ VEAKPSVYFI KFDDDFHIYV

241 YDLVKTS

Fig.6 Protein sequences of MOB-related proteins and spindlin. The peptides which were used for protein identification are underlined.

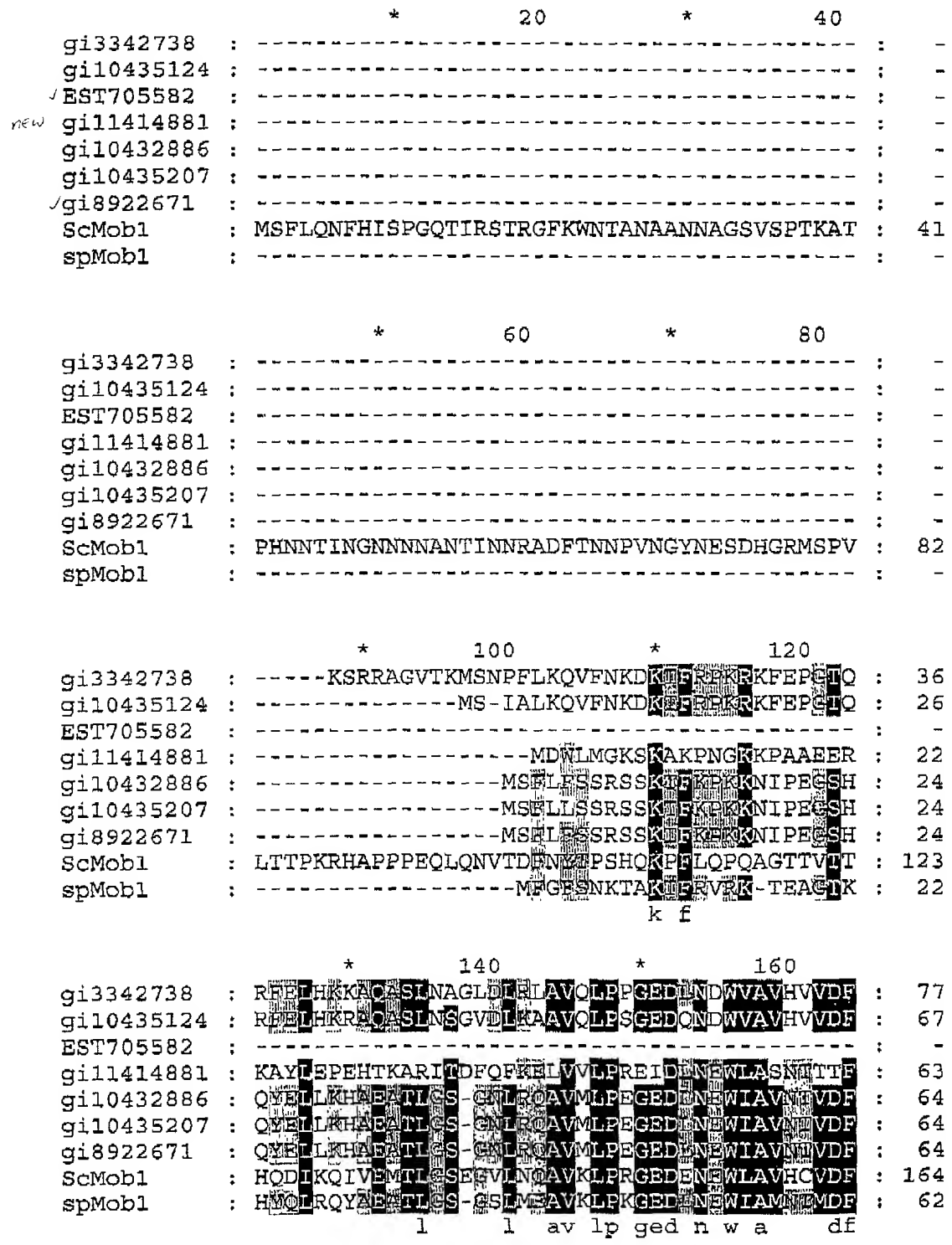

gi3342738
gi10435124
EST705582
gi11414881
gi10432886
gi10435207
gi8922671
ScMob1
spMob1
MSFLQNFHISPGQTIRSTRGFKWNTANAANNAGSVSPTKAT
PHNNTINGNNNNANTINNRADFTNNPVNGYNESDHGRMSPV
KSRRAGVTKMSNPFLKQVFNKDKTFRRPKRKFEPGTQ
MS-IALKQVFNKDKTFRRPKRKFEPGTQ
MDWLMGKSKAKPNGKKPAAEER
MSFLFSSRSSKTFKPKKKNIPEGSH
MSFLLSSRSSKTFKPKKKNIPEGSH
MSFLFSSRSSKTFKPKKKNIPEGSH
LTTPKRHAPPPEQLQNVTDFNWTPSHQKPFLQPQAGTTVTT
MFGFSNKTAKTFRVRK-TEAGTK
RFELHKKAQASLNAGLDLRLAVQLPPGEDLNDWVAVHVVDF
RFELHKRAQASLNSGVDLKAAVQLPSGEDQNDWVAVHVVDF
KAYLEPEHTKARITDFQFKELVVLPREIDLNEWLASNITTF
QYELLKHAEATLGS-GNLRQAVMLPEGEDLNEWIAVNTVDF
QYELLKHAEATLGS-GNLRQAVMLPEGEDLNEWIAVNTVDF
QYELLKHAEATLGS-GNLRQAVMLPEGEDLNEWIAVNTVDF
HQDIKQIVEMTLGSEGVLNQAVKLPRGEDENEWLAVHCVDF
HMQLRQYAEATLGS-GSLMEAVKLPKGEDLNEWIAMNTMDF

FIGURE 7

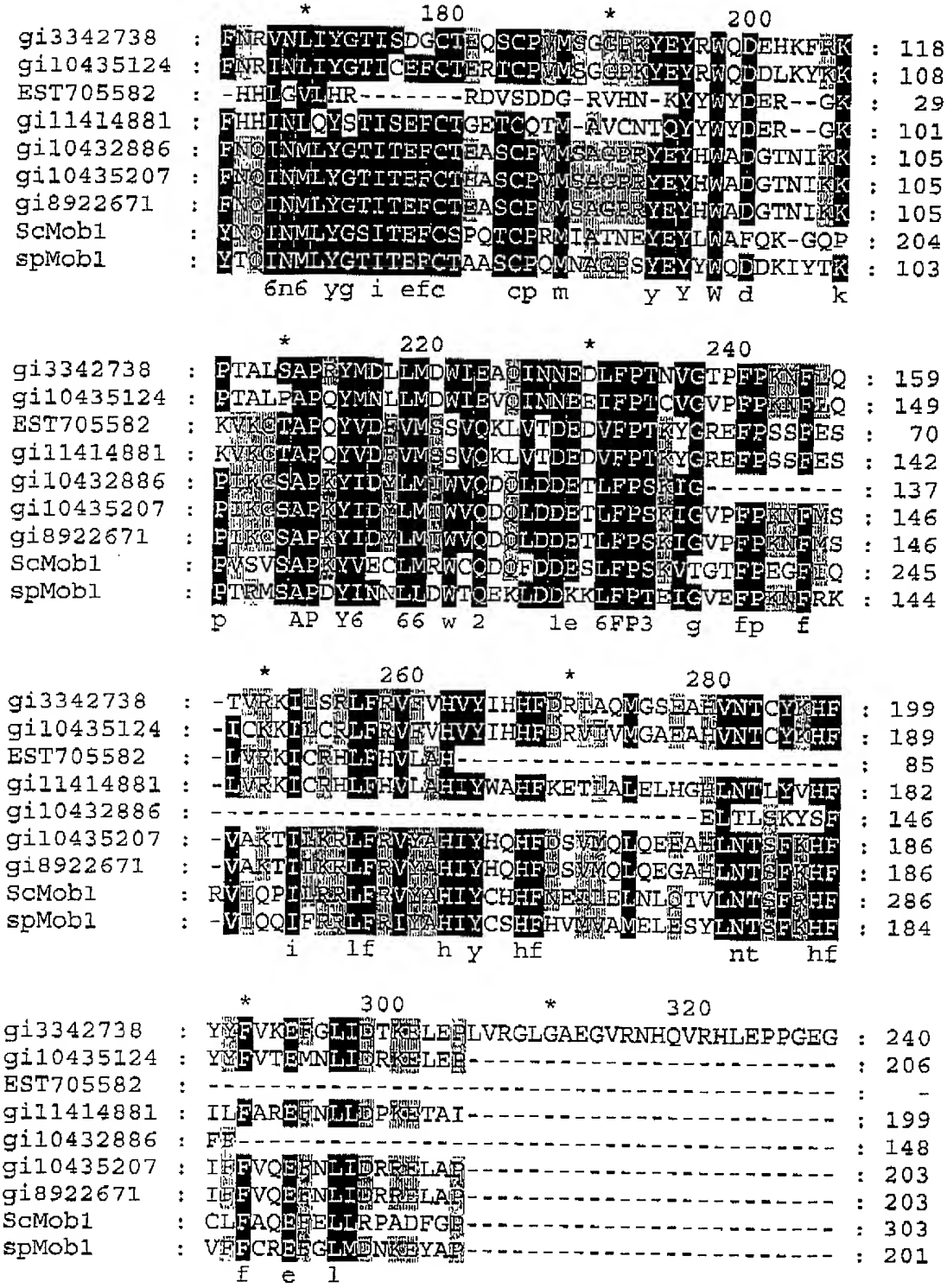


FIGURE 7 (continued)

```
                 *       340          *       360
gi3342738   : PPSRALKELHEIRNCLMKCISLYLEDEAQTPTPLSPPGLGM : 281
gi10435124  : -----LKEMTS-RMCH------------------------- : 216
EST705582   : ---------------------------------------- :   -
gi11414881  : -----MDDLTEVLCSGAGGVHSGGSGDGAGSGGPGAQNHVK : 235
gi10432886  : ---------------------------------------- :   -
gi10435207  : -----LQELIEKLGSKDR----------------------- : 216
gi8922671   : -----LQELIEKLGSKDR----------------------- : 216
ScMob1      : -----LLELVMELRDR------------------------- : 314
spMob1      : -----MQDLVDSMV--------------------------- : 210

                 *
gi3342738   : SPAARPRSFP : 291
gi10435124  : ---------- :   -
EST705582   : ---------- :   -
gi11414881  : ER-------- : 237
gi10432886  : ---------- :   -
gi10435207  : ---------- :   -
gi8922671   : ---------- :   -
ScMob1      : ---------- :   -
spMob1      : ---------- :   -
```

FIGURE 7 (continued)

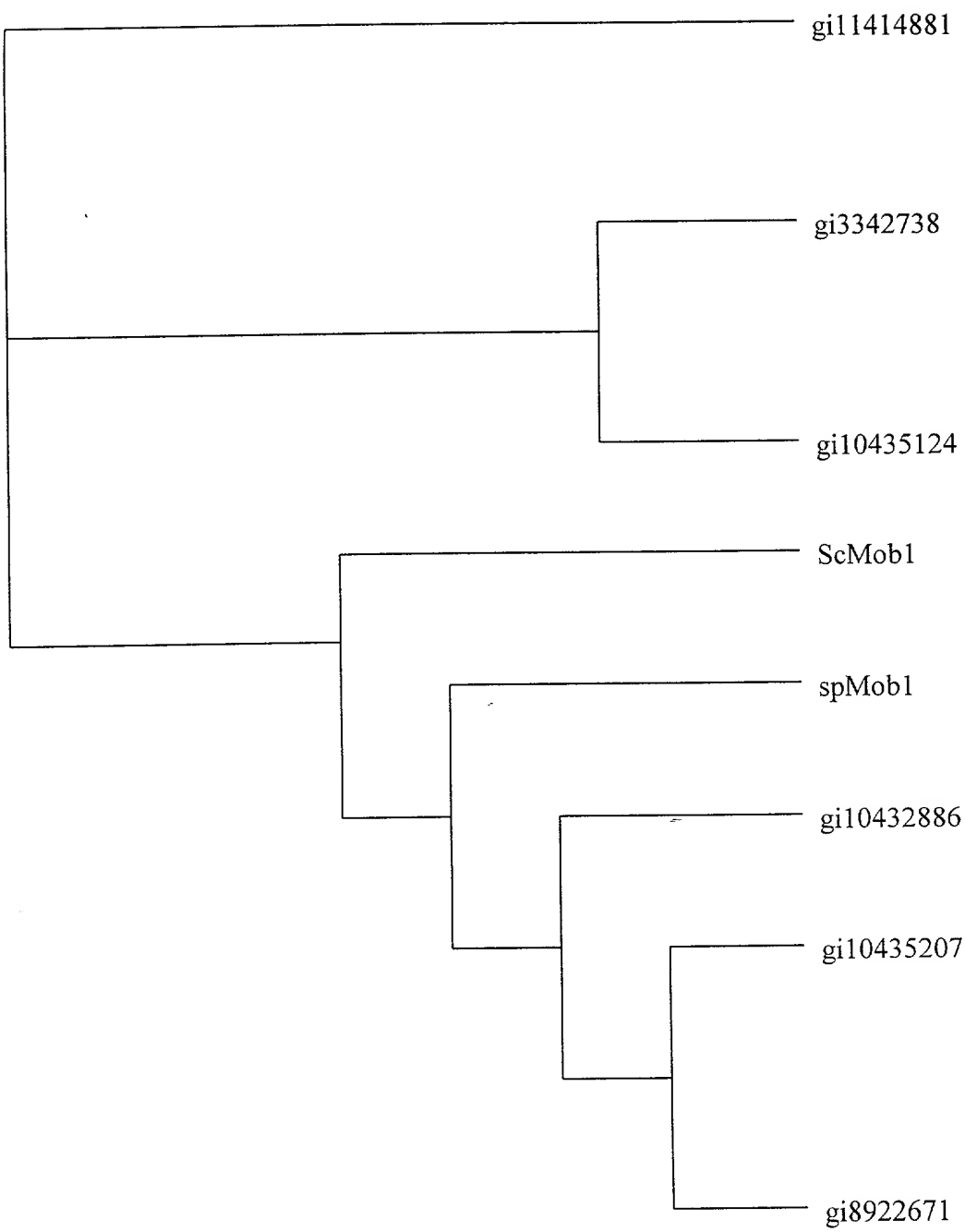


Figure 8. Mob1 related proteins.

Figure 9

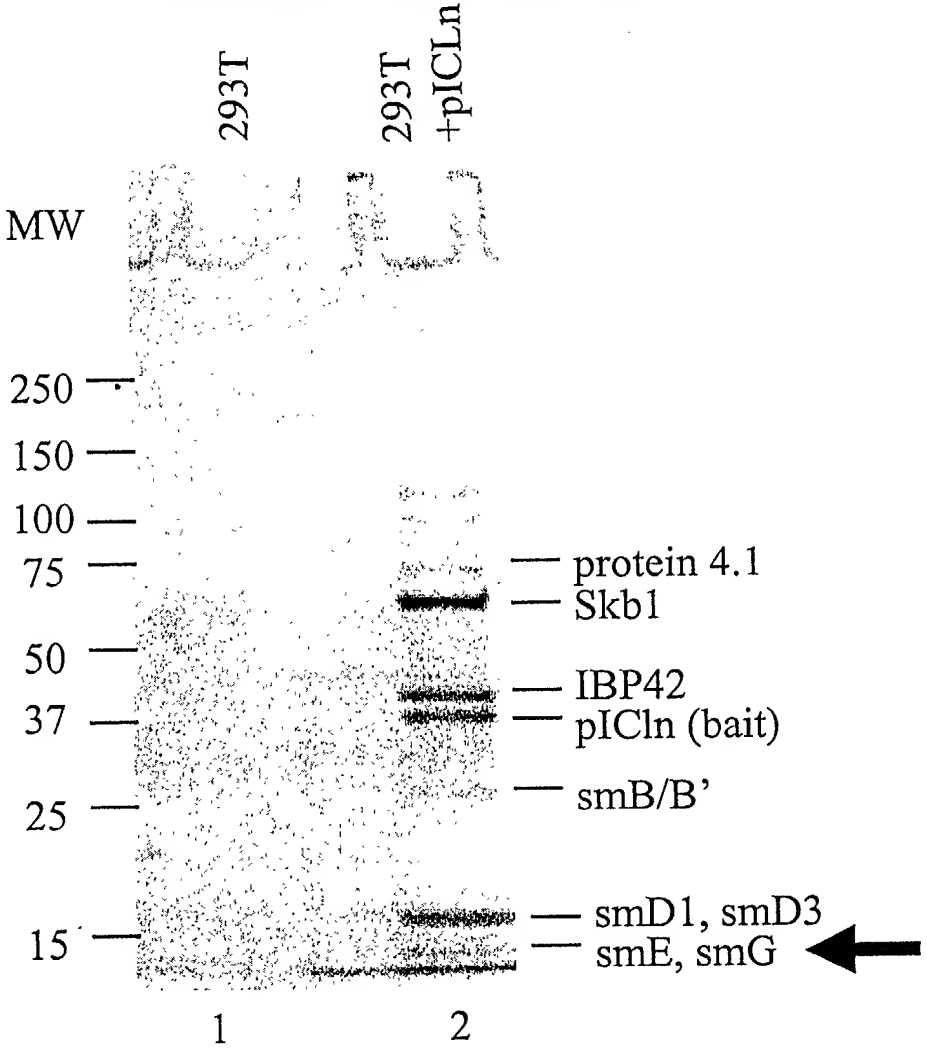


Figure 10

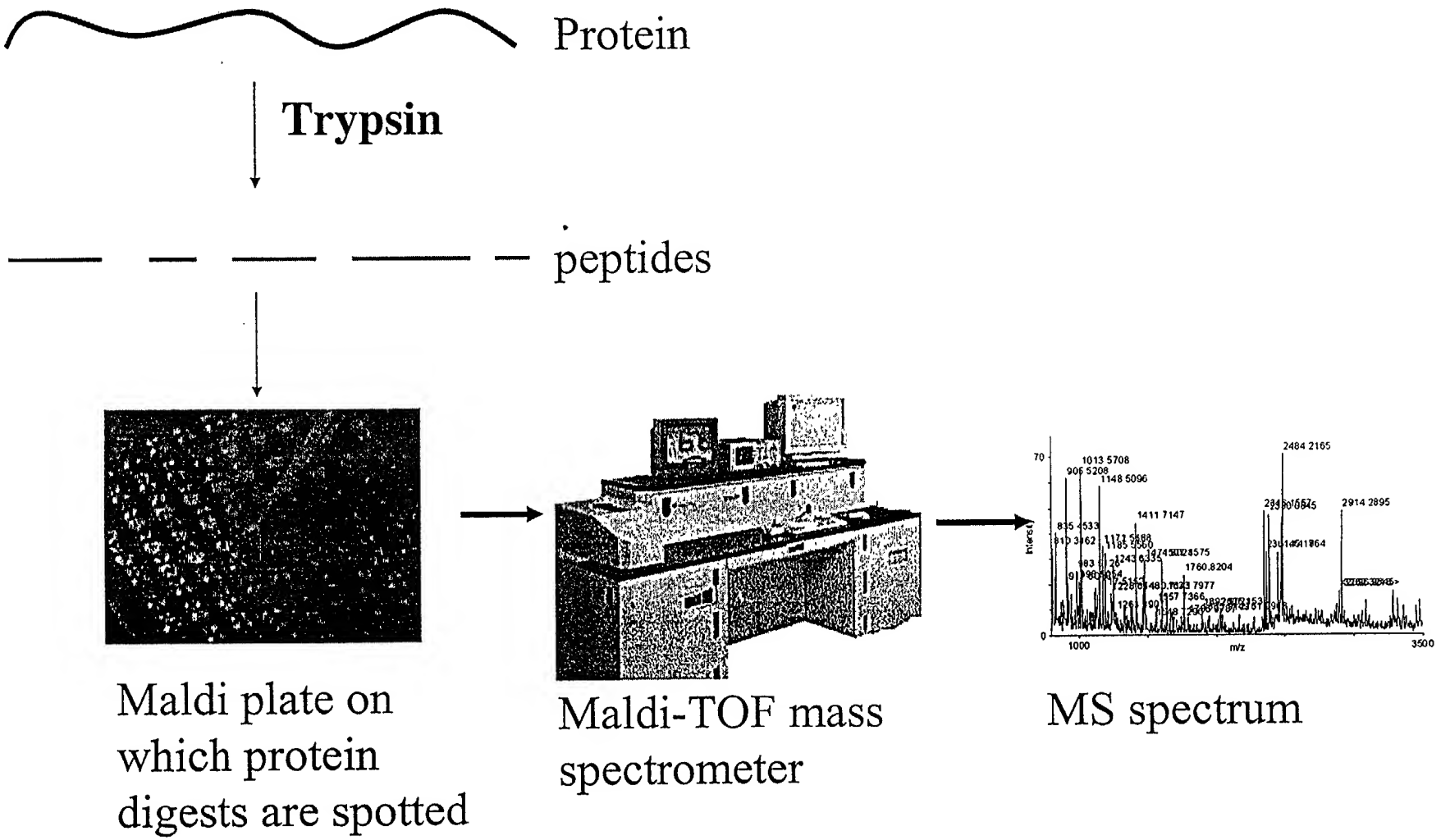


Figure 11. Protocol for the in gel digestion of proteins and the recovery of peptides for analysis by mass spectrometry.

1. Excise the spot/band of interest, cut the fragment into smaller pieces
2. Shrink the gel pieces in acetonitrile for approx. 10 min. Remove excess acetonitrile, and dry under vacuum with centrifugation (SpeedVac).
3. Cover gel pieces with 10 mM dithiothreitol (DTT) in 50-100 mM $_4HCO_3$.
4. Cool to room temp, remove DTT solution and add equal volume of 55 mM iodoacetamide in 50-100 mM NH4HCO3. Incubate for 45 min in dark with occasional stirring.
5. Rinse the gel with 50-100 µl aliquots of 50-100 mM NH_4HCO_3 for 10 min and remove excess.
6. Shrink the gel with acetonitrile for 10 min and remove excess
7. Swell the gel with 50-100 mM NH_4HCO_3, and shrink again with acetonitrile.
8. Remove excess liquid and dry down using a SpeedVac.
9. Swell gel pieces at 4 °C for 45 min in buffer containing trypsin and 50 mM NH_4HCO_3. (Approx. 5 µL/mm^2 gel). The gel pieces should just be covered. (Trypsin solution at 12.5 ng/µL is typically used for silver stained gels)
10. Digest overnight at 37 °C (or at least for 3 h).
11. Centrifuge gel pieces and collect supernatant.
12. Further extract peptides by one change of 20 mM NH_4HCO_3 followed by centrifugation and pool supernatant.
13. Further extract with three changes of 5% formic acid in 50% CH_3CN (20 min between changes) at room temp.
14. Dry sample down in SpeedVac until desired volume has been reached or to dryness.

Figure 12. Protocol for preparation of mass spectrometry samples. Desalting using ZipTip and application of sample to MALDI plate for MALDI-TOF MS analysis.

Desalting:

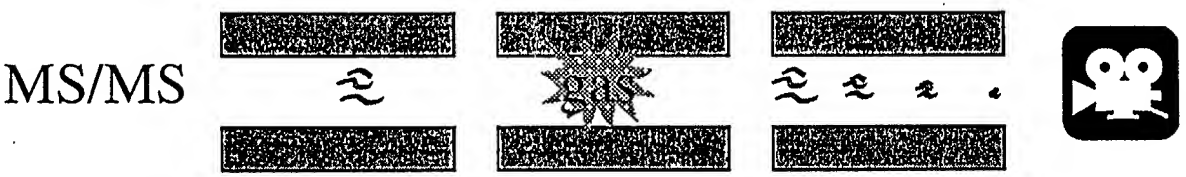
1. The sample (Step 14 in Table 1.0) should be either **a)** resuspended in 5% (v/v) methanol and 5%(v/v) formic acid or **b)** at least acidified by the addition of formic acid.
2. Wash ZipTips (Millipore) by pipetting in and out of the tip three aliquots of 60% (v/v) Methanol and 5% (v/v) formic acid solution.
3. Equilibrate the tips with 5% (v/v) methanol and 5% (v/v) formic acid
4. Extract the analytes from the sample by pipetting it up and down 10 to 20 times
5. Wash the tip with a solution of 5% (v/v) methanol and 5% (v/v) formic acid.
6. Elute the analytes off the tip using (3 ul) of a solution of 60% (v/v) methanol and 5% (v/v) formic acid.

Application to MALDI plate:

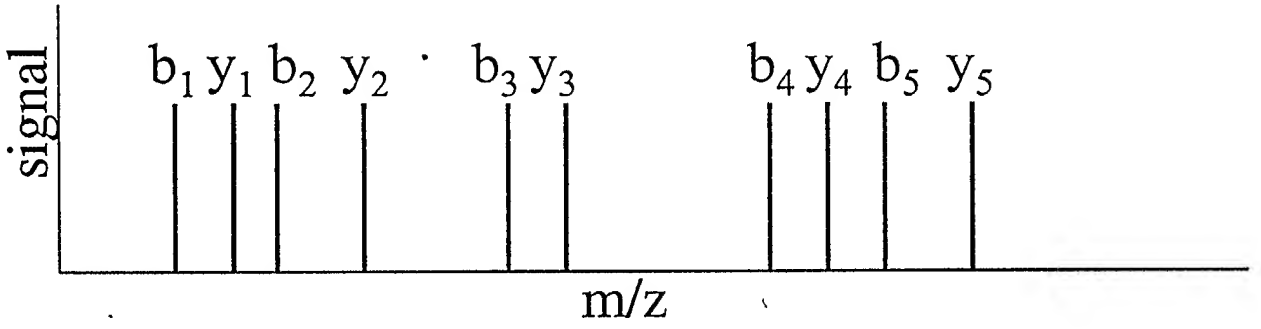
1. Prepare a fresh saturate solution of α-cyano-4-hydroxy-cinnamic acid (doubly re-crystallized) in 50% (v/v) acetonitrile 0.3% (v/v) trifluoroacetic acid.
2. Take 0.5 µl of sample (eluent in step 6 of the desalting protocol) and pipet onto one position on the MALDI plate.
3. Immediately add 0.5 µl of the saturate matrix solution (from step 1) and allow to air dry for at least 5 minutes.
4. Repeat the process for all samples.
5. Install the plate in the MALDI-TOF MS.

Figure 13

A



B



C

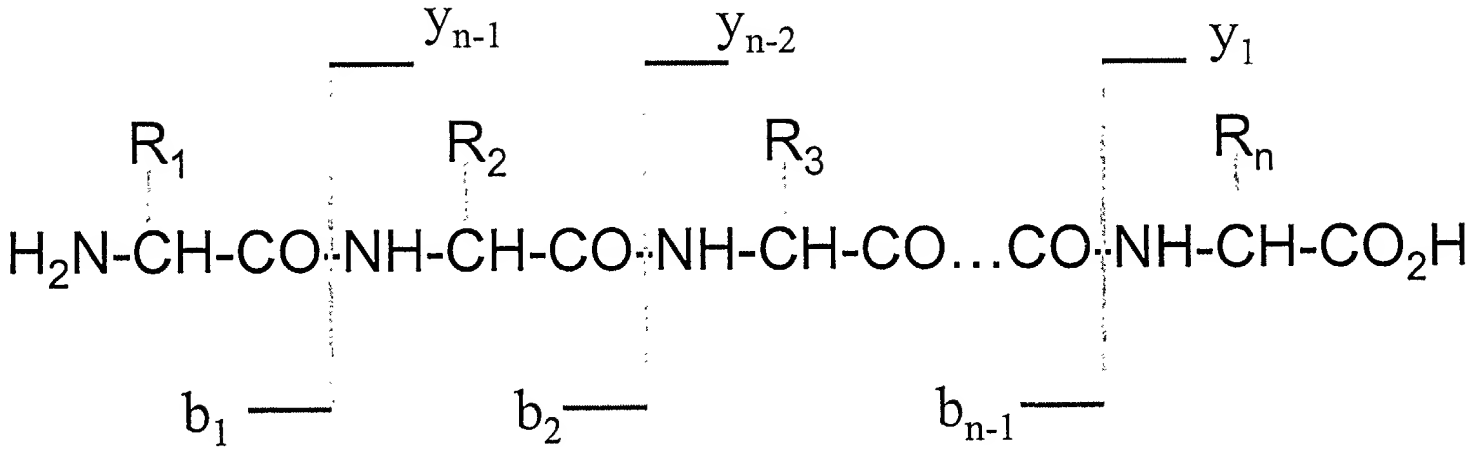
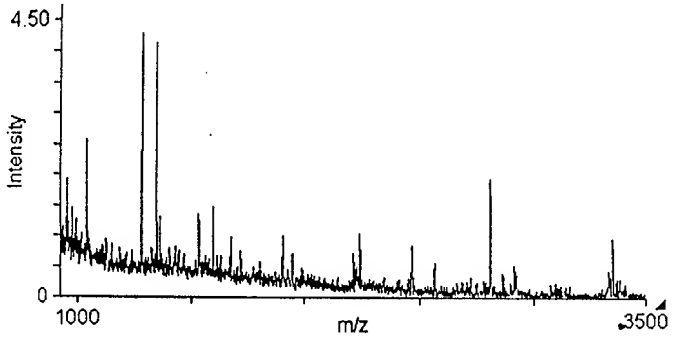


Figure 14



Extract peptide masses

973.443
1259.643
1328.702
1344.740
1593.787

Search database

Database Information:

Id3a [Rattus norvegicus]:
MKALSPVRGCYEAVCCLSERSLAIARGRGKSPSAEEP
LSLLDDMNHCYSRLRELVPGVPRGTQLSQVEILQRVI
DYILDLQVVLAEPAPGPPDGPHLPIQVREGAR**PGSSE**
RAGWDAAGLPHRVLEYLG

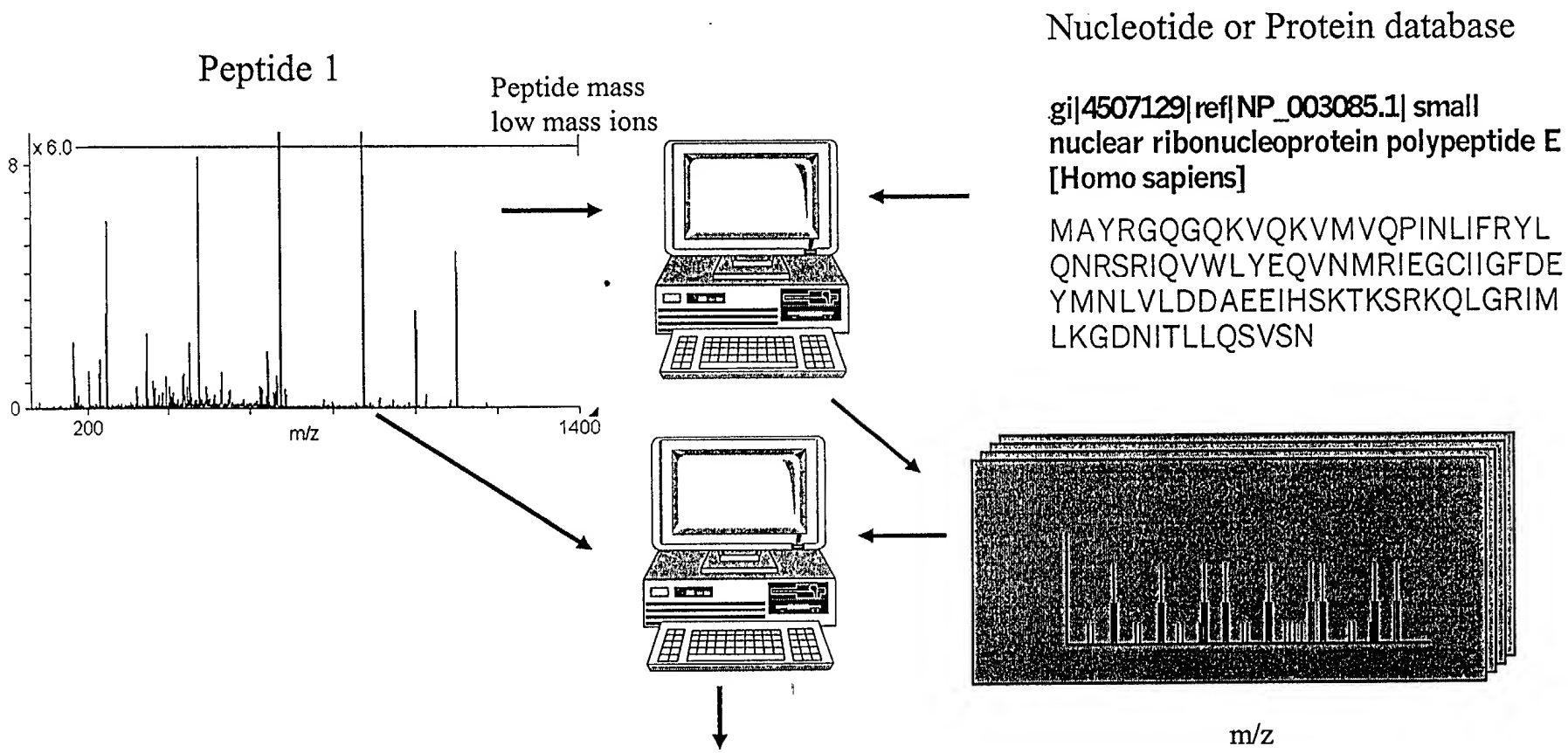
gi|4507129|ref|NP_003085.1| small nuclear ribonucleoprotein polypeptide E [Homo sapiens]
MAYRGQGQKVQK**VMVQPINLIFR**YLQNRSR**IQVW**
LYEQVNMRIEGCIIGFDEYMNLVLDDAEEIHSKTKS
RK**QLGRIMLKGDNITLLQSVSN**

Y342_METJA:
MRWLTPFGMLFISGTYYGLIFFGLIMEVIHNALISLVL
AFFVVFAWDLVLSLIYGLRFVKEGDYIALDWDGQFP
DCYGLFASTCLSAVIWTYTDSLLLGLIVPVIIVFLGKQ
LMRGLYEKIKS

Identification based on # of matching masses

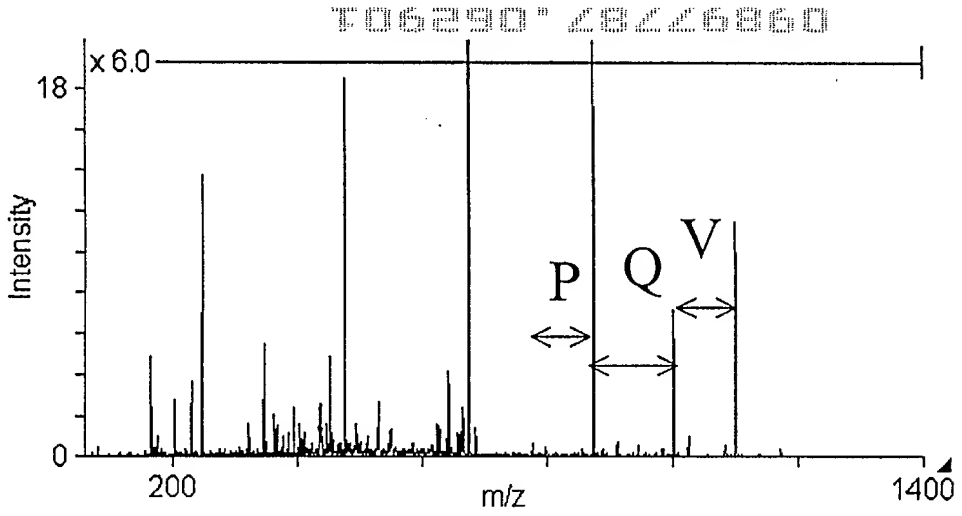
Protein identity: small nuclear ribonucleoprotein polypeptide E (smE)

Figure 15



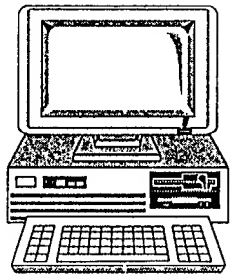
Peptide: VM^{ox}VQPINLIFR
Protein identity: small nuclear ribonucleoprotein polypeptide E (smE)

Figure 1 6



Extract peptide mass and sequence tag

Peptide mass:1345.7
(775.48) PQV (1099.66)



Nucleotide or Protein database

gi|4507129|ref|NP_003085.1| small nuclear ribonucleoprotein polypeptide E [Homo sapiens]

MAYRGQGQKVQKVMVQPINLIFRYL
QNRSRIQVWLYEQVNMRIEGCIIGFDE
YMNLVLDDAEEIHSKTKSRKQLGRIM
LKGDNITLLQSVSN

Match tag and masses to the database

Peptide: VM^{ox}VQPINLIFR
Protein identity: small nuclear ribonucleoprotein polypeptide E (smE)

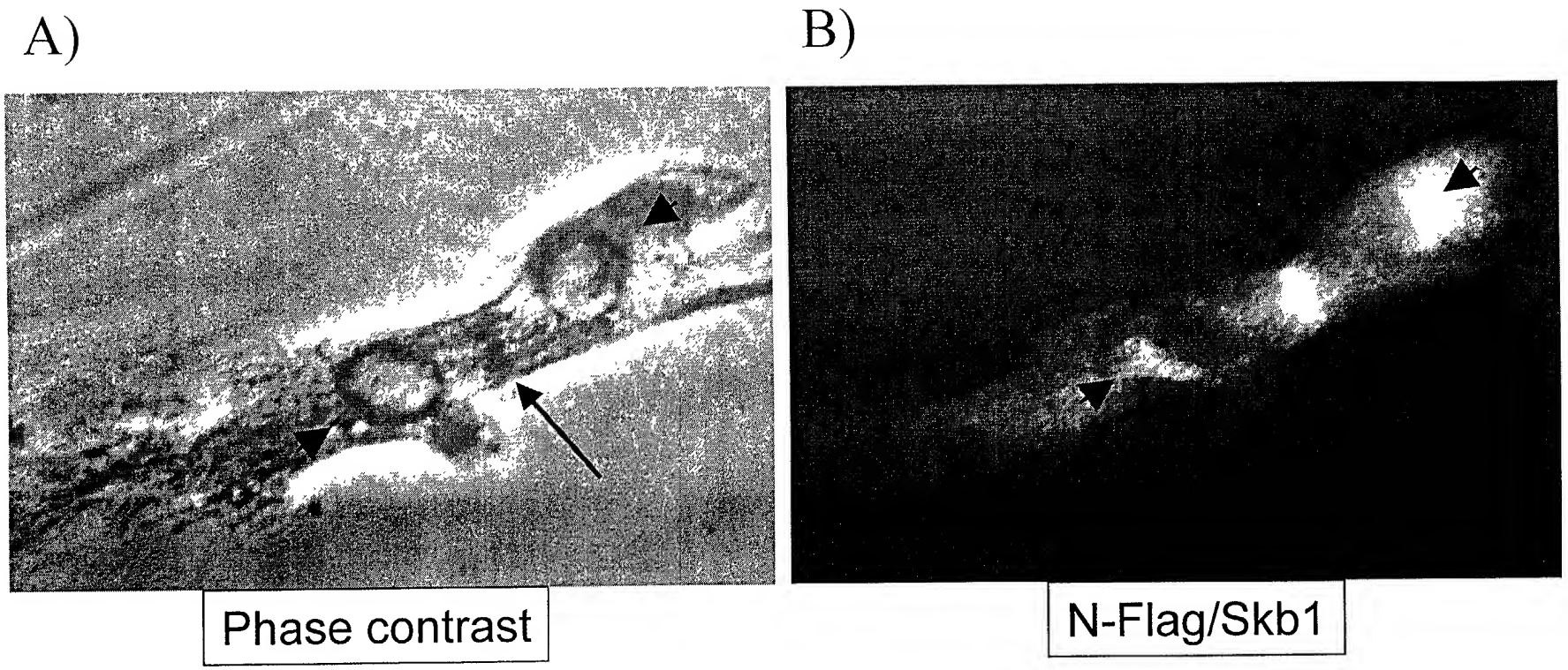


Figure 17 . Localization of Human Skb1 at Telophase. Human normal dermal fibroblasts were transfected with N-terminal FLAG tagged Skb1 and stained with FITC conjugated anti-FLAG antibody. A) shows the phase contrast image and B) shows the FITC staining. Human Skb1 localizes to the cleavage furrow (large arrow), and possibly at the spindle poles (small arrows), during telophase.

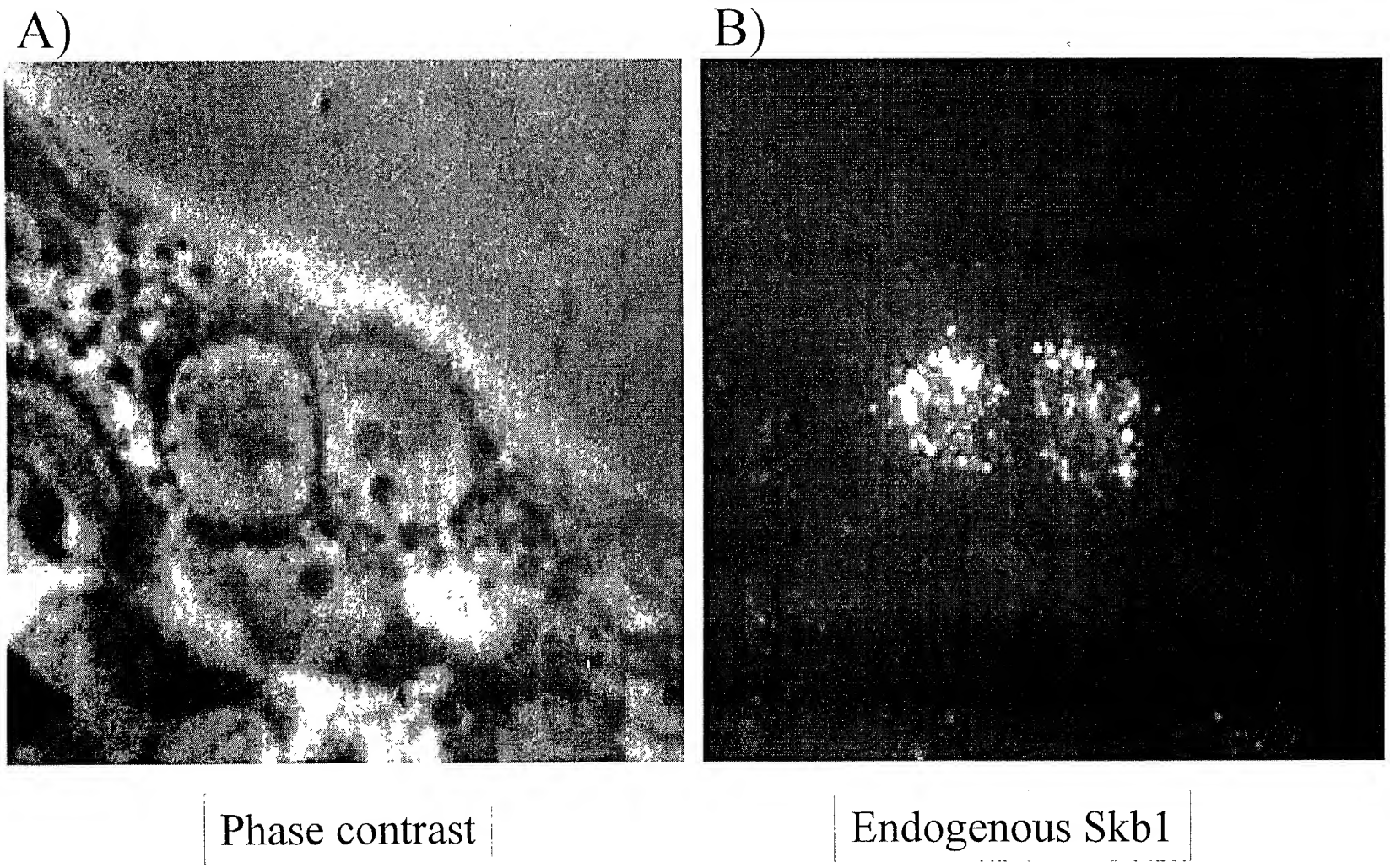


Figure 18. Nuclear staining of interphase HepG2 cells with anti-Skb1 antibody.
HepG2 cells show a speckled nuclear staining pattern which does not colocalize with the nucleolus, but resembles that of nuclear speckles.

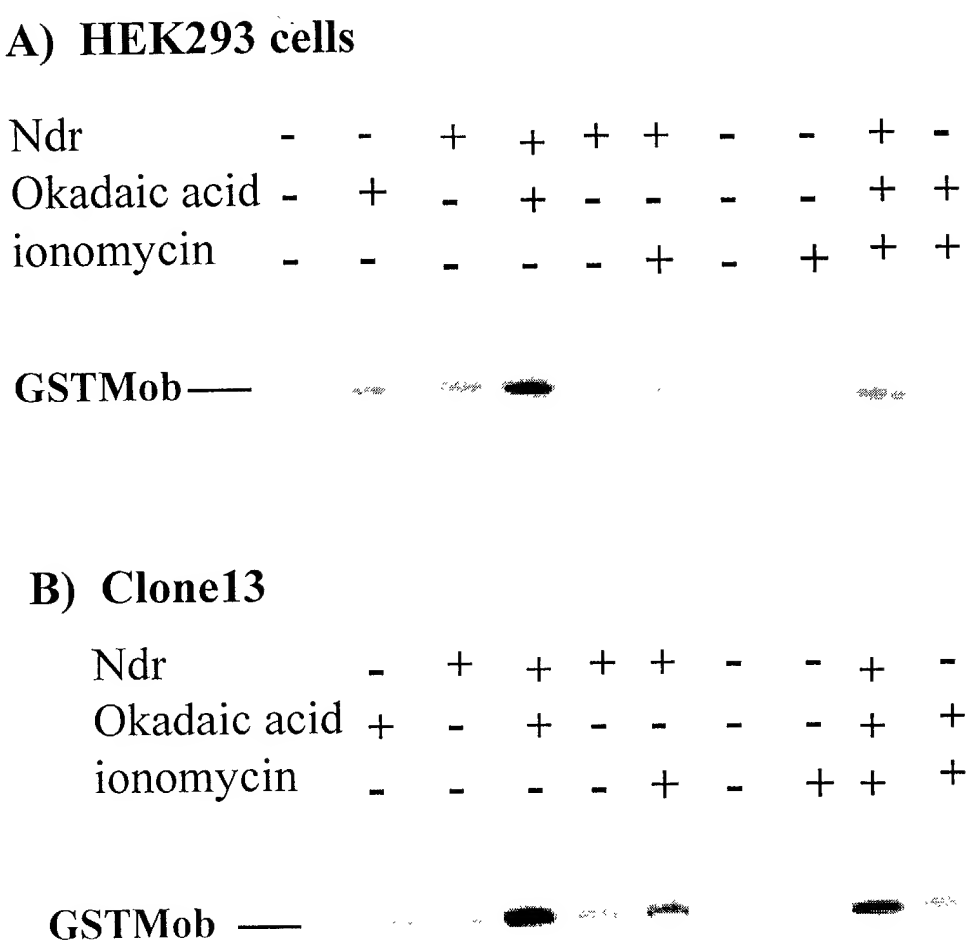


Figure 19. Co-expression of Grf2 may modulate Ndr kinase activity. A) HEK293 cells or B) HEK293 cells stably expressing GRF2 (clone 13 Line) were transfected with FLAG tagged Ndr. Cells were treated with Okadaic acid for 1hour and/or ionomycin for 5 minutes prior to cell lysis and Ndr immunoprecipitation, as indicated by +. Immunoprecipitated proteins were incubated with γ^{32}P ATP and a GST-Mob protein substrate for 1hr at 30C.Proteins were resolved by gel electophoresis and exposed on a phosphorimager screen. GSTMob is phosphorylated by Ndr isolated from Okadaic acid treated cells,and this phosphorylation increases in the presence of GRF2. Likewise, GST-Mob was more heavily phosphorylated in cells treated with both ionomycin and okadaic acid, when GRF2 was co-expressed with Ndr.

```
                    *         20          *         40
gi13543922 : ---------------------------------------- :   -
AF258661   : ---------------------------------------- :   -
sudD       : ---------------------------------------- :   -
RIO1       : ---------------------------------------- :   -
HssudD     : MDLVGVASPEPGTAAAWGPSKCPWAIPQNTISCSLADVMSE :  41
FLJ11159   : ---------------------------------------- :   -

                    *         60          *         80
gi13543922 : ----------------------------------MSRVV :   5
AF258661   : ---------------------------------------- :   -
sudD       : --------------------------------MSSDSTTQA :   9
RIO1       : ---------------------------------------- :   -
HssudD     : QLAKELQLEEEAAVFPEVAVAEGPFITGENIDTSSDLMLAQ :  82
FLJ11159   : ----------------------------------MGKVNV :   6

                    *         100         *         120
gi13543922 : PGQFDDADSSDSENRDLKTVKEKDDILFEDLQDNVNENGEG :  46
AF258661   : ---------------------------------------- :   -
sudD       : ASPAEGLNPSHTYVPNKGYANEDGAVPAMAGQDLTPEDEDY :  50
RIO1       : ---------------------------------------- :   -
HssudD     : MLQMEYDREYDAQIRREEKKFNGDSKVSISFENYRKVHPYE : 123
FLJ11159   : AKLRYMSRDDFRVLTAVEMGMKNHEIVPGSLIASIASLKHG :  47

                    *         140         *         160
gi13543922 : EIEDEEEEGYDDDDDDWDWDEG-VGKLAKGYVWNGGSNPQA :  86
AF258661   : ---------------------------------------- :   -
sudD       : EGDEYYDDIFEEELDEGDFNSSNPADLTKAYNRQRRVNELA :  91
RIO1       : ---------------------------------------- :   -
HssudD     : DSDSSEDEVDWQDTRDDPYRPAKPVPTPKKGFIGKGKDITT : 164
FLJ11159   : GCNKVLRELVKHKLIAWERTKTVQGYRLTNAGYDYLALKTL :  88

                    *         180         *         200
gi13543922 : NRQTSDSSSAKMSTPADKVLRKFENKINLDKLNVTDSVINK : 127
AF258661   : ---------------------------------------- :   -
sudD       : ADPNAPKWTYPKTNTQKPTVNTYASVDDEIKSLTRHAAKIK : 132
RIO1       : --------MSLEDKFDSLSVSQGASDHINNQLLEKYSHKIK :  33
HssudD     : KHDEVVCGRKNTARMENFAPEFQVGDGIGMDLKLSNHVFNA : 205
FLJ11159   : SSRQVVESVGNQMGVGKESDIYIVANEEGQQFALKLHRLGR : 129
```

FIGURE 20

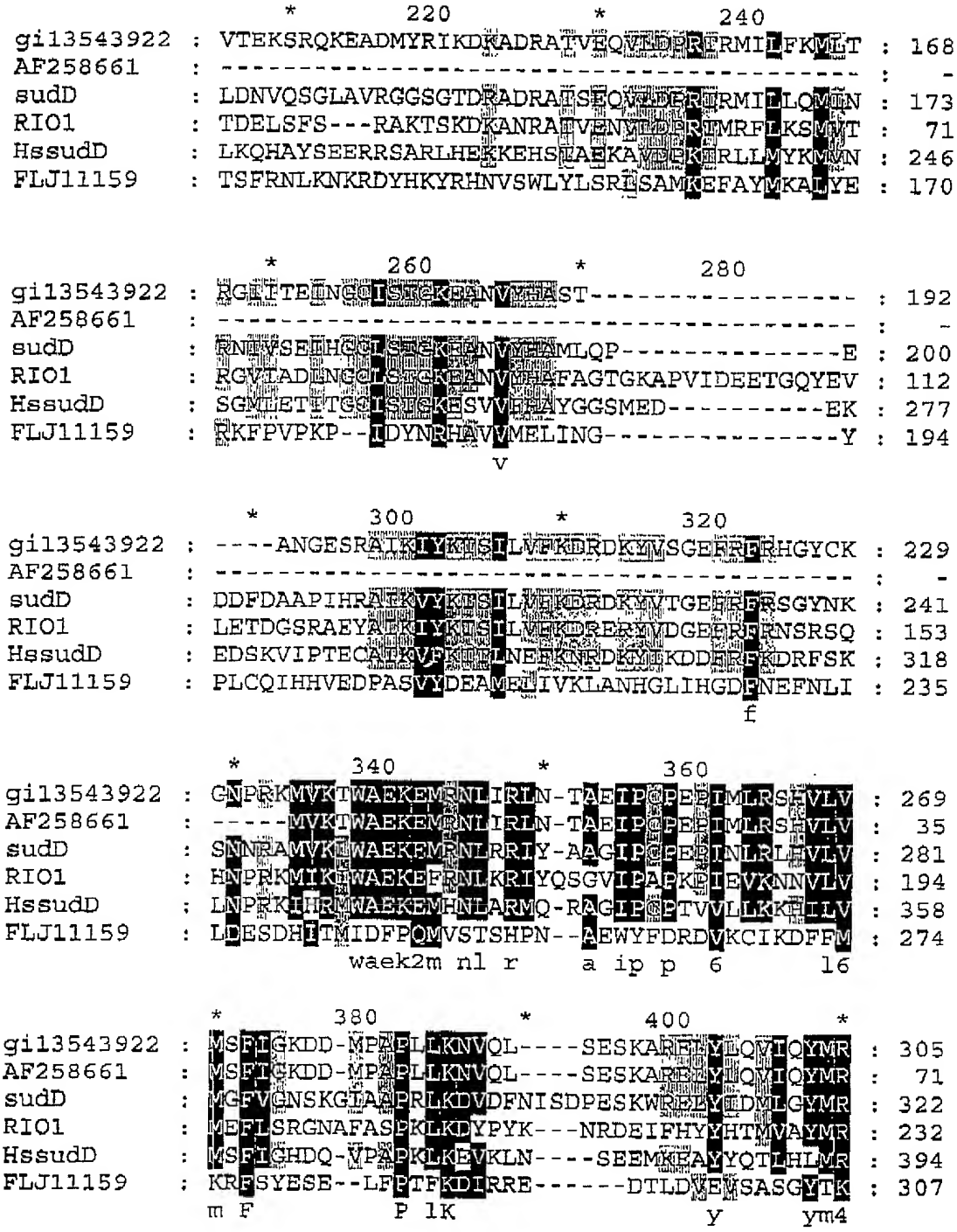


FIGURE 20 (continued)

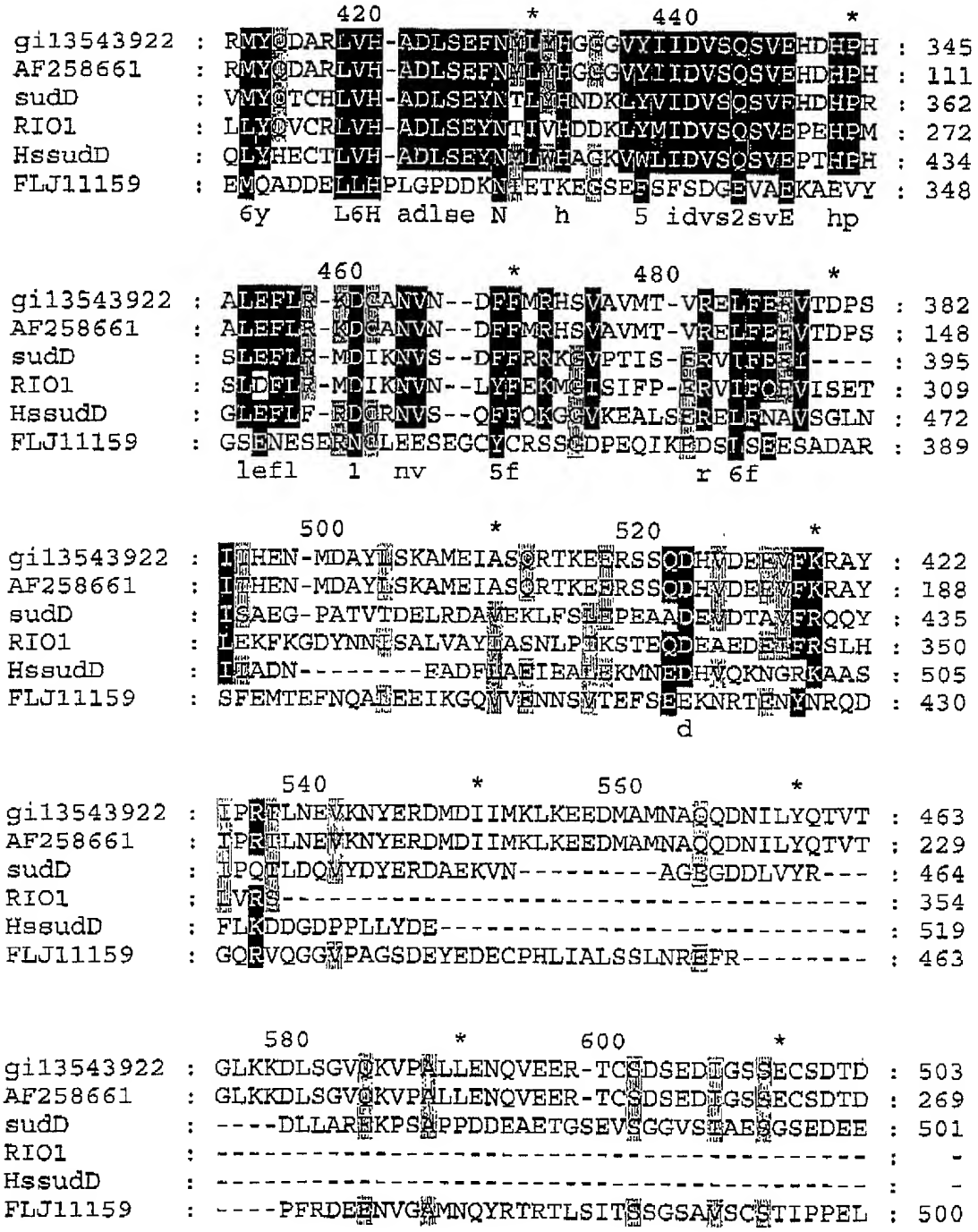


FIGURE 20 (continued)

```
                620         *       640         *
gi13543922  :  SEEQGDHARPKKHTTDPDIDKKERKKMVKEAQREKRKNKIP  :  544
AF258661    :  SEEQGDHARPKKHTTDPDIDKKERKKMVKEAQREKRKNKIP  :  310
sudD        :  ERDPFEKKPPRGKRFEDKESKKEHKNKVKEEKREKRANKMP  :  542
RIO1        :  ----------------------------------------  :    -
HssudD      :  ----------------------------------------  :    -
FLJ11159    :  VKQKVKRQLTKQQKSAVRRRLQKGEANIFTKQRRENMQNIK  :  541

                660         *
gi13543922  :  KHVKKRKEKTAKTKKGK  :  561
AF258661    :  KHVKKRKEKTAKTKKGK  :  327
sudD        :  KHIKKRLVSSSSRKRK-  :  558
RIO1        :  ----------------  :    -
HssudD      :  ----------------  :    -
FLJ11159    :  SSIEAASFWGE------  :  552
```

FIGURE 20 (continued)